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Cyclic GMP-linked pathway for renin secretion

ALAN R. NOBLE, RABEE A. ABU-KISHK, MARIE-ANN E. D'ALOIA, BRENT C. WILLIAMS, and DAVID J. LUSH

Department of Physiology & Pharmacology, University of Southampton, England; Department of Medicine, University of Edinburgh, Western General Hospital, Edinburgh, Scotland; and Department of Biological Sciences, University of the West of England, Bristol, England, United Kingdom

Cyclic GMP-linked pathway for renin secretion. The role of cGMP as a second messenger for renin secretion is contentious. This was investigated using a superfused collagenase-dispersed rat kidney cortex cell preparation devoid of indirect influences on renin secretion. Nitroprusside, atriopeptin II and 8-Br-cGMP all increased renin release but the dose-response relationships were biphasic. At low dose ranges there was a positive correlation between increasing drug concentration and renin secretion, but at high drug concentrations, a negative correlation was apparent. Methylene blue, a guanylate cyclase inhibitor, also suppressed baseline renin release at 10^{-5} and 10^{-6} M, but stimulated release at 10^{-3} M. Using mid-range drug concentrations, the cGMP specific phosphodiesterase inhibitor MB22948 potentiated renin release in response to nitroprusside and 8-Br-cGMP. Inhibition of guanylate cyclase with either methylene blue or LY83583 attenuated renin release in response to nitroprusside, but, as expected, had no effect on 8-Br-cGMP induced release. We conclude that, under physiological conditions, cGMP is a stimulatory second messenger for renin release. This activity is mimicked at low dose ranges by 8-Br-cGMP, nitroprusside and atriopeptin II. In response to high doses of these drugs an unknown inhibitory pathway is activated and this opposes, in a dose-related manner, the stimulatory actions of cGMP for renin release.

Nitric oxide (NO) and nitrovasodilator drugs activate soluble guanylate cyclase and thus increase the formation of cGMP. There are conflicting reports concerning the direct effects of this pathway in the regulation of renin secretion from juxtaglomerular (JG) cells. *In vivo*, drugs which alter cGMP formation may alter many aspects of renal function, vascular tone, baroreceptor sensitivity and neural activity. However, studies using *in vitro* preparations have also failed to provide consistent results. Some authors [1, 2], using isolated perfused kidneys, report that NO and cGMP have a stimulatory effect on renin release, whereas others [3–5], on the basis of studies using kidney cortex slices, suggest that the NO-cGMP pathway is inhibitory for renin release. As both kidney slices and isolated perfused kidneys retain an intact microanatomy, drug responses of JG cells may still be mediated through indirect effects on adjacent cell populations. A study carried out using primary cultures of mouse JG cells [6] has shown a biphasic effect: NO-linked inhibition of renin release in the first hour followed by a stimulation over the succeeding 20 hours. The time course of this study is, however, quite different to the acute responses studied by other groups.

We used a superfused collagenase-dispersed rat kidney cortex cell preparation [7] to investigate acute cGMP-linked renin secretion. Indirect influences mediated by attached cell populations were thus eliminated. Two very important aspects of our study were that, in contrast to other studies, a wide range of drug doses was used, and that a superfusion protocol was used. The data obtained supports the concept that cGMP is fundamentally a stimulatory second messenger for renin release, but bell-shaped drug dose-response relationships were obtained. High doses of drugs associated with increased intracellular [cGMP] caused markedly submaximal increases in renin release. This suggests the progressive activation, by high drug doses, of an inhibitory pathway for renin secretion which opposes the cGMP-linked stimulation of release.

Methods

Details of the methods used have been published previously [7]. Briefly, fresh rat kidney cortex tissue was chopped and digested with collagenase. Cells were harvested three times during the digestion to prevent prolonged exposure to the enzyme. They were then filtered successively through gauzes of 250 μm, 100 μm and 60 μm mesh to ensure separation of undigested fragments of tissue. This technique also led to an enrichment of the JG cell preparation by elimination of a significant proportion of renal tubule fragments. Approximately 10^7 cells were layered onto Biogel P2 in each of four PTFE chambers and superfused with Krebs Ringer bicarbonate buffer containing 0.2% glucose and 0.2% bovine serum albumin (Fraction V; Miles). Cells were allowed to equilibrate for 90 minutes and then 40 five-minute superfusate samples were collected. During this time each cell preparation was challenged on four occasions for 10 minutes with a drug dose. Parallel columns, using different aliquots of the same cell preparation, were used to test agonist ± antagonist, thereby eliminating variation in cell responsiveness between preparations as a factor when interpreting results.

Renin concentration in the superfusate was measured by radioimmunoassay of angiotensin I generated during incubation with excess renin substrate prepared from the plasma of sheep six days after bilateral nephrectomy. The same substrate preparation was used for all of the samples in any one series of experiments.

Mean basal renin release before and after drug stimulation was assigned 100% and mean renin release in response to stimulation was expressed as a response ratio (RR), that is, as a percentage of baseline release. A paired Student's *t*-test was used to compare drug responses with basal renin release.

The sources of the drugs used were as follows: 8-Br-cGMP (Sigma), sodium nitroprusside (NP) (BDH) atriopeptin II (APII)

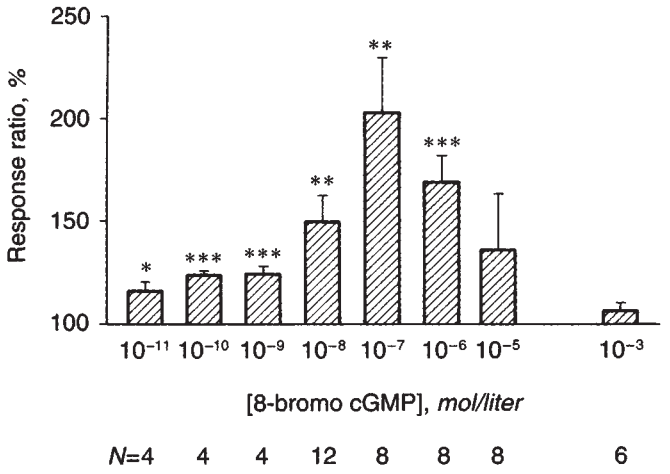

Fig. 1. *Wide range dose-response relationship for 8-Br-cGMP induced renin release.* Results are shown as mean ± SEM.

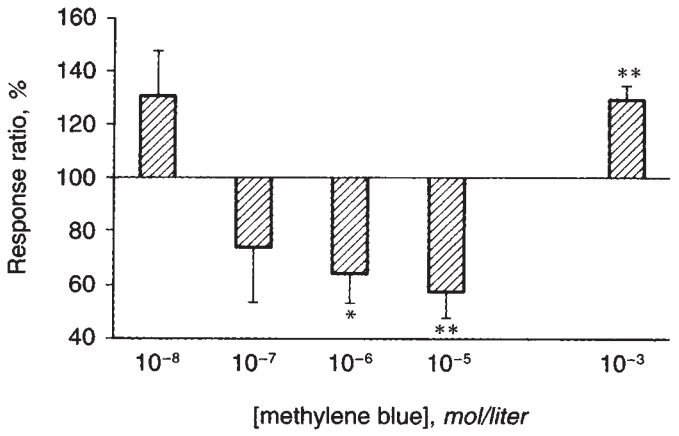

Fig. 2. *Effect of a range of concentrations of methylene blue on basal renin release.* Results are shown as mean ± SEM.

(Sigma), methylene blue (MeB) (Michrome), LY83583 (Eli Lilly), MB22948 (Rhone-Poulenc).

Results

Figure 1 shows responses to a range of concentrations (10^{-11} to 10^{-3} M) of the cell permeant analogue 8-Br-cGMP. Between 10^{-11} M and 10^{-7} M there is a direct dose-response relationship with increasing [8-Br-cGMP] causing progressively greater increases in renin secretion. With 10^{-7} M 8-Br-cGMP, mean release of renin is 102.5% above baseline ($P < 0.01$). At higher [8-Br-cGMP] an inverse relationship with renin release was found, and no significant increase in renin release occurred with the two highest doses (10^{-5} M and 10^{-3} M) of the drug. Mean renin release with 10^{-3} M 8-Br-cGMP was only 6.3% above basal release.

A similar pattern of response to the 8-Br-cGMP data was obtained in studies using NP and APII (results not shown). NP, studied over the range 10^{-10} to 10^{-3} M produced peak stimulation of renin release at 10^{-7} M (RR = 179.2%; $P < 0.001$) but at 10^{-3} M NP the mean increase in renin release, although still significant, was only 16.2% ($P < 0.05$) above baseline. Responses to APII, which also increased renin release, were studied over the dose range 10^{-14} M to 10^{-7} M. A peak response occurred at 10^{-10} M APII (R.R. = 174.5%; $P < 0.01$) but at 10^{-7} M APII the increase in renin release was only 23.7% above the baseline ($P < 0.01$).

Studies using MeB, an inhibitor of soluble guanylate cyclase also yielded a biphasic pattern of response (Fig. 2). With 10^{-6} M MeB (RR = 73.5%; $P < 0.05$) and with 10^{-5} M MB (RR = 57.4%; $P < 0.01$), basal renin release (100%) was significantly reduced but 10^{-3} M MeB increased renin release (RR = 128.7%; $P < 0.01$). No statistically significant changes were recorded with the two lowest concentrations of MeB used.

The stimulatory role for the cGMP pathway in the regulation of renin release was confirmed in a further series of experiments (data not shown). The cGMP-specific phosphodiesterase inhibitor MB22948 (10^{-5} M) potentiated renin release in response to NP (10^{-7} M) by 214.1% ($P < 0.001$) and in response to 8-Br-cGMP (10^{-6} M) by 156.6% ($P < 0.05$). MeB (10^{-5} M) attenuated the response to NP (10^{-7} M) by 50.1% ($P < 0.001$) but, as expected, did not have any effect on responses to 8-Br-cGMP (10^{-8} M). LY83583 (10^{-6} M), a drug which lowers intracellular [cGMP], reduced the stimulation of renin release by NP (10^{-7} M) by 73.7% ($P < 0.01$) and also had no effect on 8-Br-cGMP (10^{-8} M) stimulated renin release.

Discussion

The data presented primarily support the hypothesis that cGMP is a stimulatory second messenger for renin release but also provide evidence of a second, inhibitory pathway activated by high drug doses. The experimental results were obtained using a superfused collagenase dispersed rat kidney cortex cell preparation in which secondary effects on renin secretion mediated via changes in smooth muscle tone, neural activity or kidney tubular function were eliminated. In addition, the use of a superfusion technique avoids the potential effects on renin secretion of the accumulated secretory and metabolic products present in static cell incubation protocols.

The analogue 8-Br-cGMP (Fig. 1), NP and APII stimulated renin release at all doses used. Responses to 8-Br-cGMP and NP were potentiated by the cGMP specific phosphodiesterase inhibitor MB22948. The increased renin secretion produced by NP was markedly attenuated by the guanylate cyclase inhibitor MeB and by LY83583, a drug which reduces intracellular [cGMP] partly by suppressing guanylate cyclase [8]. As would be expected, MeB and LY83583 did not affect the increase in renin release produced by 8-Br-cGMP. A stimulatory role for cGMP in the control of renin release has previously been proposed on the basis of studies using the isolated perfused rat kidney preparation [1, 2].

Some authors, using kidney cortex slice preparations, have found evidence that cGMP is an inhibitory second messenger for renin release [3–5]. We propose from the present study that there are two separate and opposing pathways, a stimulatory pathway activated by low concentrations of drugs and an inhibitory pathway activated at high drug dose levels. Dose-response relationships in this study had two distinct phases, a positive correlation between low range drug doses and renin release and a negative correlation between high range drug doses and renin release. This is shown for 8-Br-cGMP in Figure 1, but very similar data were obtained for NP and APII. Further evidence of an inhibitory pathway involving cGMP is shown in Figure 2. The soluble

guanylate cyclase inhibitor MeB, at 10^{-6} M and 10^{-5} M, inhibited basal renin release, suggesting blockade of a stimulatory pathway for renin. However, 10^{-3} M MeB caused significant stimulation of renin secretion, suggesting the existence of a cGMP-linked inhibitory pathway.

It seems likely that, under physiological conditions, the major role for cGMP is as a stimulatory second messenger for renin and that the inhibitory pathway modulates this role, perhaps via an increase in intracellular [Ca^{++}].

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Reprint requests to Dr. A.R. Noble, Department of Physiology & Pharmacology, University of Southampton, Bassett Crescent East, Southampton SO9 3TU, England, United Kingdom

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